# An Underwater Bioluminescence Assessment Tool (U-BAT)

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## LONG-TERM GOALS

Our long-term objective for Phase II of our research is the construction of four Underwater Bioluminescence Assessment Tool (U-BAT) commercial prototypes that have been fully characterized in the laboratory and field, thereby paving the way for widespread use and acceptance of the U-BAT to measure bioluminescence by oceanographers and the Navy. WET Labs will transition the technology, developed at UCSB, into a commercially available version that is small, light weight, platform-adaptable and couple it with already proven key bio-optical instruments to provide a single system for wide-scale, cost effective, full water column profile biological discrimination. The envisioned U-BAT will directly address Naval survey and tactical operations in providing a visibility and vulnerability assessment for deployed assets and potential threats. By broadening the use of bioluminescence (BL) measurements U-BAT will significantly increase general understanding of the roles of BL in oceanic biodynamics. This work directly addresses ONR topic # N05-T026 for the need to transition new and novel BL sensing technologies from the research to the commercial realm in order to enable a more comprehensive quantification of the spatial and temporal variability of biogeochemical complexity in coastal and oceanic ecosystems.

## OBJECTIVES

The primary objective for the STTR Phase II project is to produce four commercial prototypes of the Underwater Bioluminescence Assessment Tool (U-BAT), a general purpose commercial bathyphotometer for biological assessment of natural waters evolved from the Multipurpose Bioluminescence Bathyphotometer (MBBP-G3) technology developed at UCSB. Our goals for this phase of the STTR Phase II project were: 1) to build 2 U-BAT prototypes from the assimilated mechanical, electrical and control code designs of the MBBP-G3 technology and to incorporate design improvements defined by a core group of bioluminescence researchers, 2) to test the performance of U-BAT prototype-1 in the laboratory and field, 3) to determine the utility of integrating other Inherent Optical Properties (IOPs) with the U-BAT sensor, 4) to provide calibration technology and methodology for measurement traceability, and 5) to build 2 U-BAT prototype-2s, evolved from the U-BAT prototype-1 which include design modifications made to simplify commercial manufacturing and serviceability.

## APPROACH

A key asset for the design and development components of the final U-BAT product is the involvement of a core group of researchers in the field of bioluminescence. Drs. James Case (UCSB), Mark Moline (Cal Poly), Edith Widder (Ocean Research & Conservation Association), Steve Haddock

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(MBARI), Mike Latz (Scripps), and Mark Geiger (NAVO) compose our academic and Naval partners. Continued involvement of our partners in prototype testing will play a crucial role toward the development of the commercial U-BAT. As the MBBP-G3 technology is evolved into the commercial product, frequent design review meetings are essential to communicate and incorporate design changes to ensure that the U-BAT will provide the naval and oceanographic community with a single system for wide-scale, cost effective, full water column profile biological discrimination. Extensive trials coupled with iterations of system modifications are critical to the overall success of the Phase II project in that they will help define the desired range and capacity for the commercial U-BAT product.

**WORK COMPLETED**

Following the build of the first two 2 U-BAT prototypes (prototype-1), both laboratory and field tests were performed and the build of the second 2 U-BAT prototypes (prototype-2) was completed. Analysis of the performance of U-BAT prototype-1 was used to provide a pathway for improving the technology used for the design of the second two U-BAT prototypes (prototype-2). A review of the performance of U-BAT prototype-1, highlighted the need for improvements in the efficiency of manufacturing, instrument serviceability, and reduction in sensor weight. During the Phase II award period the following was accomplished:

*1. Presentation of U-BAT at Ocean Sciences (February 2008) and Ocean Optics (October 2008)*
Results from development of U-BAT prototype-1 and U-BAT prototype-2 design were presented at Ocean Sciences 2008, Orlando, FL and will be presented at Ocean Optics XIX in October 2008 in Tuscany, Italy. Posters for both conferences and an extended abstract for OOXIX highlight the following laboratory and field results: 1. U-BAT is linear over each gain setting, 2. U-BAT is able to capture data on the short-term time evolution of organism flash kinetics (60 Hz), 3. Long-term profile data shows that U-BAT is capable of resolving BL signals on vertical scales << 1m resolution (1 Hz average). 4. Bioluminescence, in conjunction with other IOPs, can provide a more complete picture of spatial and temporal variability of biogeochemical complexity of coastal and ocean ecosystems, especially the response of the planktonic community to environmental fluctuations.

*2. Completion of a monochromatic validation light wand*
A validation light source provides the user with technology for measurement traceability. The light source provides a fixed light flux, within the mid-range of PMT detection, and is centered at a common wavelength of peak light emission for marine bioluminescent organisms (470nm). The light flux of the light wand is determined at WET Labs. A Labsphere CDS-1100 will be used to provide traceable for each light source provided to the user. With this, the user can track the drift in the U-BAT signal due to possible PMT drift or due to the effect of biofouling. The validation light source is keyed to ensure consistent results.

*3. Data analysis from Avila profiler (ongoing)*
During August 8-14, 2007 side-by-side comparison of U-BAT prototype-1, MBBP-G2, and MBBP-G3 was conducted at the Cal Poly pier, Avila, CA. Results show that the mechanically stimulated bioluminescence of U-BAT and MBBP-G3 are strongly correlated, (U-BAT = 1.44 * MBBP-G3 + 7.70e+10 ($r = 0.90$). Discrete water samples were collected during this study for analysis. Identification of bioluminescent phytoplankton is complete and identification of bioluminescent zooplankton is underway. Results show that there is no significant difference between the capture efficiency of MBBP-G3 and U-BAT prototype-1. Both phyto- and zooplankton dataset will be

incorporated as part of bioluminescence signal analysis study, conducted by Moline. Since August 2007, the autonomous profiler has continued to take profiles every ½ hour.

*4. Design modifications*

Mechanical design modifications were made to improve manufacturability and serviceability of the U-BAT. One significant improvement has been to mold U-BAT parts. The following U-BAT parts are molded: pump and flow impellers, helical ramp at intake, detection chamber, PMT faceplate, PMT baffle, PMT dome and helical exhaust baffle. Acrylic with 20% loading of titanium oxide was chosen as the detection chamber material and is > 95% reflective between 430-700 nm. Molding the PMT dome, PMT baffle and acrylic face plate as a single component improved the depth rating of U-BAT, from 200 to 600 m. Use of a helical exhaust baffle may reduce the effect of biofouling and improve the user's ability to service U-BAT following deployment. An interior MCBH-6-MP wet-matalble connector connects the pump-motor with control electronics in the PMT dome, reducing the number of cables.

*5. Presentation of U-BAT technology at NAVOCEANO (September 10-12, 2008)*

With the invitation of the Ocean Optics group, Ray Pluhar and Megan Natter, WET Labs Senior Research Associate, Cristina Orrico, traveled to Stennis Space Center, MS and gave a presentation on the U-BAT technology. In attendance were individuals, who support various components of the optics group research including: operational engineers and research scientists involved in data collection, data processing and modeling. On hand was a U-BAT for the group to inspect and take apart. The operation engineers were impressed with the size, weight and maintenance requirements of U-BAT. Also presented were general specifications for the U-BAT software system, currently under development. The optics group made several suggestions that will assist in software development to meet the needs of the Navy and general research user.

*6. Preparation for ship-of-opportunity deployment (October-November, 2008)*

As a result of the September 2008 meeting and the Navy's interest in testing the performance of U-BAT, preparations are underway to participate in a side-by-side deployment of U-BAT with OTiS which is one of commonly used NAVOCEANO tools. WET Labs will provide Ray Pluhar with U-BAT and battery pack to install onto the profiler package containing OTiS. Discussions are underway to install U-BAT into the underway system on the TAGS-60 Pathfinder class ships. Although, U-BAT is not a sealed instrument, installation in either a sealed pipe or sea chest is being considered.

*7. Software specifications defined*

Front end software specifications have been defined. The Graphic User Interface (GUI) will allow the user to view and record data from the Underwater Bioluminescence Assessment Tool (U-BAT). At power up, U-BAT outputs comma delimited ASCII data. Contained in the data record are the following: instrument serial number, record number from start up, calibration coefficients for the three gain settings, 1 Hz averaged BL (with calibration coefficients applied), pump RPM, flow RPM, and 1-60 raw PMT counts. The software will include the following attributes: real-time 1 Hz data view (with applied calibration coefficients), real-time 60 Hz data view, user interface to save incoming data. It will also automatically apply offsets obtained from calibration light wand to the factory calibration coefficients.

## RESULTS

All four prototype builds have been completed and a build for five additional units are underway as we move toward full commercial production. The primary embodiment of the U-BAT prototype-1 sensor system was compiled from detailed knowledge of the MBBP-G3 design technology and input from our core group of research collaborators, especially Cyril Johnson, James Case and Mark Moline. Modifications were made to prototype-2 to make its design more effective for commercialization and improve serviceability.

To decrease the weight of the sensor, excess material was removed from the exterior and also provides a sleek look (Figure 1). Since some of the U-BAT components required long machine times, we chose to mold several U-BAT components. The following components are molded: pump and flow impellers, helical ramp at intake, detection chamber, acrylic faceplate, PMT baffle, PMT dome, and helical exhaust baffle. To reduce the number of cables needed for U-BAT, the pump-motor connector was moved inside the pressure housing and connects to the control housing with a MCBH-6-MP wet-matealble connector.

A key design modification is the improved depth rating. U-BAT prototype-1 and MBBP-G3 were rated to 200 m, however by molding the acrylic face plate, PMT baffle and PMT dome as a single part the depth rating was increased to 600 m. The MBBP-G3 acrylic face plate failed at 750 psi. Molding the once separate components into a unified part, improves the mass of material available to withstand an increase in pressure and reduces the possibility of air pockets which could lead to a structural failure at depth.

In the U-BAT prototype-1, water traveled over two 90 degree horizontal waves which acted as light baffles. A design improvement in U-BAT prototype-2 is that the exhaust port is helical with 3 turns. The new exhaust port offers less flow restriction, acts as an external light baffle, and can be easily removed and cleaned during instrument service. At the exit to U-BAT protype-2, the helical light baffle is protected by a copper frame that may act as an antifoulant and reduce the effect of biofouling.

Although the detection chamber and exhaust components are easily cleaned, if biofouling were extensive, the detection chamber could easily be replaced and the sensor recalibrated. This is an advantage especially when considering the effect of biofouling on the detection chamber during long-term moored deployments. Since molded parts are more cost-effective to produce, they can be more readily available to stock for replacement if needed.

***Figure 1: U-BAT commercial design.***

Since the U-BAT detection chamber is designed after an integration sphere, the reflectance of the material that is used is essential for appropriately sensing bioluminescence. The detection chamber, PMT baffle and PMT dome in U-BAT prototype-1 were machined from virgin Teflon. In U-BAT prototype-2, they are molded from 20% titanium dioxide loaded in acrylic. Several materials were tested to ensure that a changing the material used for the detection chamber did not change the characteristics needed for bioluminescence sensing. Using a Shimadzu 2501 UV/VIS spectrophotometer and integration sphere with sold sample attachment the percent reflectance was determined for Teflon, zinc dioxide, and titanium dioxide. Samples of various thickness and finish were tested. It was found that titanium oxide is greater than 95% reflective from 430-700 nm. The emission spectrum of marine bioluminescence organisms fall well within the range of detection (Figure 2).

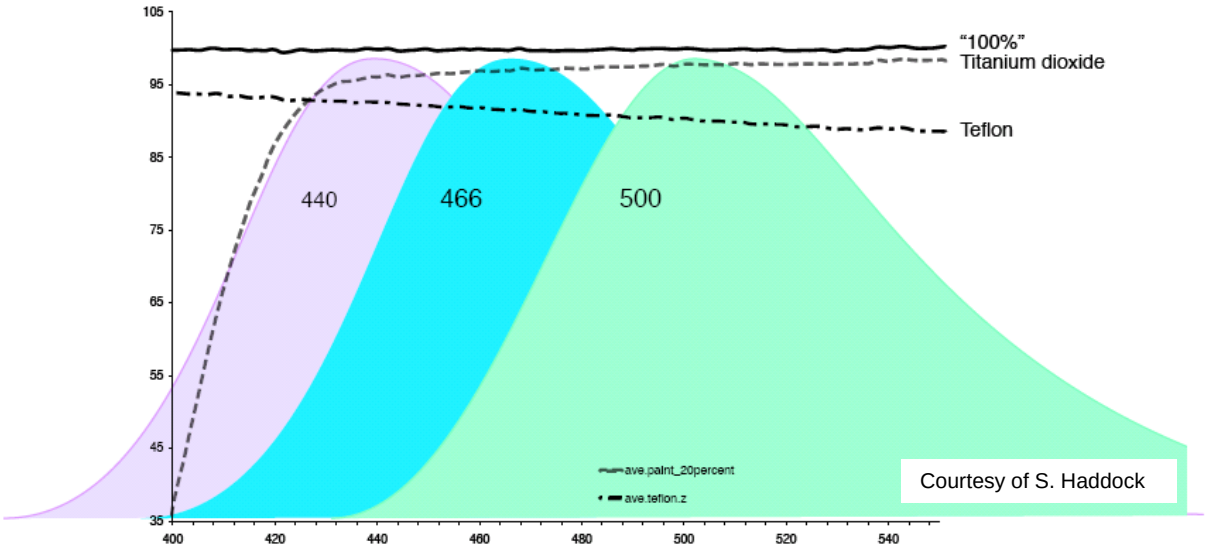



***Figure 2: Comparison of the spectral percent reflectance between titanium dioxide and Teflon with the emission spectrum of bioluminescent marine organisms, overlaid. The color of the emission curve corresponds to the peak emission wavelength of the bioluminescent organism.***

A monochromatic validation light source is complete (Figure 3). The validation light source is powered and controlled from the U-BAT command line. The wavelength of the light source is centered at 470 nm, the peak emission wavelength of *Pyrocystis fusiformis*, and the output flux is in the mid-

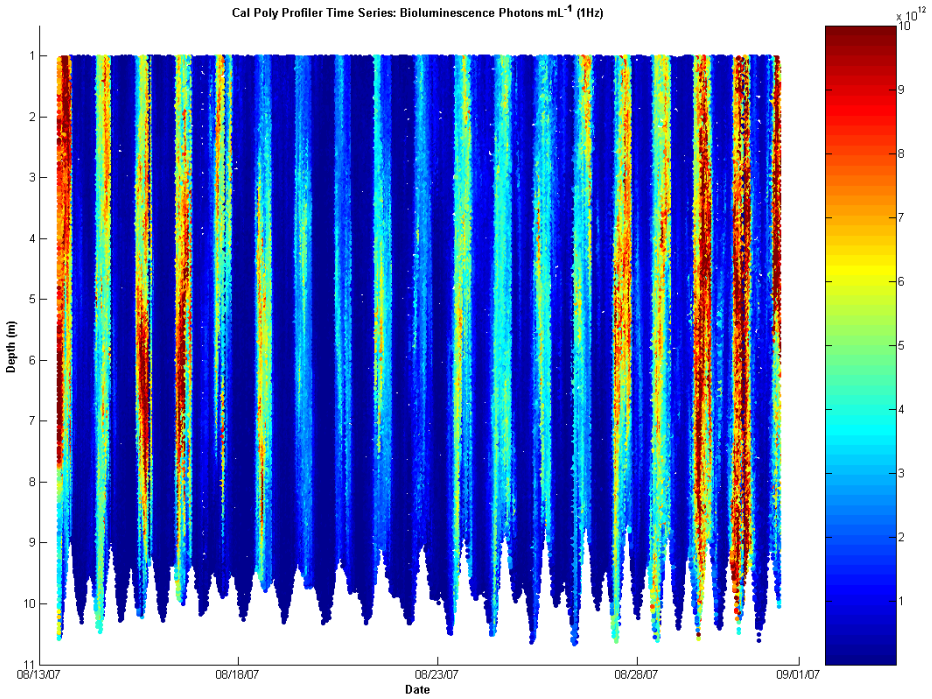
range of the PMT sensing capability. A 100 W light bulb is five orders of magnitude brighter than the light flux of the light source. The validation light source will allow the user to track sensor drift due to possible PMT degradation or biofouling. As the front end software continues to develop, the validation light wand will be used to automatically obtain PMT response offsets which will be recorded for tracking purposes and applied to the factory provided calibration coefficients. Development of this technology is paramount to the success of U-BAT and provides individual researchers with a traceable common denominator measurement against which they can compare results.

The design of this sensor is very unique in that 3 gain settings permit wide dynamic range, over 6 orders of magnitude, and the sensor is equipped with a method to protect the PMT from possible damage if the sensor were exposed to bright light. In U-BAT prototype-1 and MBBP-G3, the unit automatically shut power to the PMT if it were exposed to bright light for more than 1 second (approximately $3e^{12}$ photons $s^{-1}$). However analysis of data collected during a 2004 deployment in Santa Barbara Channel, conditions were such that in the presence of a bioluminescent red-tide (*Lingulodinium polyedrum* ) bioluminescence was so bright that the MBBP-G3 automatically shut down. Therefore, the gain settings of U-BAT prototype-2 were modified to be a factor of 10 apart which broadens the range of bioluminescence sensing capability to 7 orders of magnitude.

***Figure 3: Monochromatic light source that provides a traceable tool to track possible sensor drift due to PMT degradation or due to the effect of biofouling. Light flux falls within the mid-range of PMT detection.***

From August 8-14, 2007 side-by-side comparison of U-BAT prototype-1, MBBP-G2, and MBBP-G3 was conducted at the Cal Poly pier, Avila, CA. The U-BAT was installed on the Cal Poly autonomous profiling system along with the following instruments: WET Labs fluorescence and turbidity sensor (ECO-FLNTU), WET labs C-star turbidity sensor, Sea Tech scattering sensor (LSS), Sea-Bird conductivity, temperature and depth (CTD) and plankton capture nets. Multi-parameter profiles were collected every ½ hour and discrete water samples were collected and preserved approximately every hour to test for possible differences between MBBP-G3 and U-BAT capture efficiency. Since August, side-by-side bioluminescence profiles of MBBP-G2, MBBP-G3 and U-BAT have continued every ½ hour.

During the August deployment of U-BAT prototype-1, bioluminescence was inversely correlated with chlorophyll and turbidity (Figure 4). Comparison of results from August 22-27 show chlorophyll fluorescence and turbidity increased to 22-27 μg $L^{-1}$ and 1.2-2 NTU, respectively, whereas bioluminescence was at a minimum ($4e^{12}$-$6e^{12}$ photons $mL^{-1}$). However bioluminescence is greatest between August 13-17 and again August 27-31, with a maximum bioluminescence of $10e^{12}$ photons $mL^{-1}$, when fluorescence and turbidity are at a minimum. During August 13-17, discrete water samples were collected to determine the dominant bioluminescent organism that contributed to the bulk bioluminescence signal. The most common species found was *Protoperidinium leonis*, known to be a harmful algal bloom (HAB) associated with diarrheic shellfish poisoning. The concentration of *P. leonis* was approximately 9,727 ± 1454 cells $mL^{-1}$. Other common bioluminescent species found were: *Dinophysis acuminata, D. fortii* and *Ceratium divaricatum.* Side-by-side comparison of phytoplankton collected from the exhaust of U-BAT-prototype-1 and MBBP-G3 show that there is now significant difference between the capture efficiency of sensors. Results show that the mechanically stimulated bioluminescence of U-BAT and MBBP-G3 are strongly correlated, (U-BAT = 1.44 * MBBP-G3 + 7.70e+10 (r = 0.90). Results from this study show that bioluminescence, in conjunction with other IOPs, can provide a more complete picture of spatial and temporal variability of biogeochemical complexity of coastal and ocean ecosystems, especially the response of the planktonic community to environmental fluctuations.



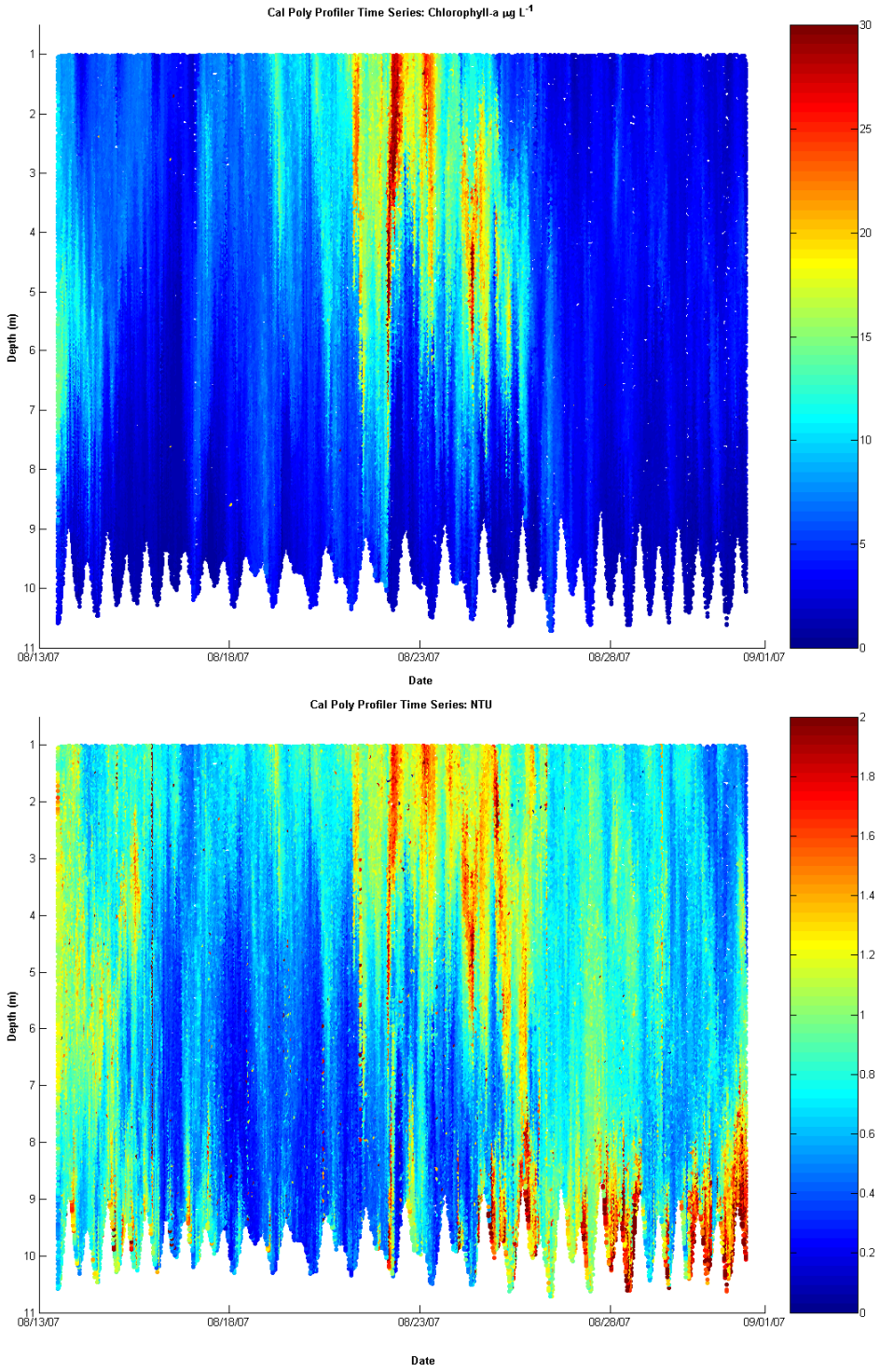



***Figure 4: Vertical profiles of (top) Bioluminescence (photons $mL^{-1}$), (middle) Chlorophyll-a ($\mu g\ L^{-1}$), and (bottom) Turbidity (NTU). Bioluminescence is inversely related to chlorophyll-a and NTU.***

## IMPACT/APPLICATIONS

While bioluminescence has long held promise as an important tool for Naval operations and biological research and monitoring, thus far there have been no commercially available tools for wide scale measurement and dissemination of results with a standard methodology. The bioluminescence research community is currently in a situation in which individual research groups tend to adopt or develop unique measurement solutions depending upon applications, available resources, and other factors. This in turn has created a scenario in which there is virtually no common denominator measurement against which individual researchers can compare results. Our goal in developing the U-BAT sensor is to ensure a common bioluminescence measurement baseline that is well characterized in terms of sensor stability, accuracy, and response and that is inter-calibrated with Naval bioluminescent standards.

We believe that the U-BAT technology will fulfill not only a DoD need, but also a need within the academic marine research realm by providing these communities with a light-weight, inexpensive bioluminescence sensor capable of accurately measuring fine-scale vertical bioluminescence potential (<< 1 m resolution). In addition, the calibration methodology defined in Option I, the U-BAT will be well characterized and intercomparable with data collected using the MBBP-G3. With special attention to the needs of the operational Navy, the U-BAT will provide a modern, commercially available instrument to collect bioluminescence data to incorporate into the Navy database, needed to assess the possible risk of flow-stimulated bioluminescence. The upcoming NAVOCEANO deployment (October, 2008) is the first opportunity for the Navy to test the performance of U-BAT and compare it OTiS, one of the Naval standards. As the U-BAT is used in the field to collect bioluminescence data, our academic partners will begin to develop data end products using the combination of optical and bioluminescence measurements to 1) better assess biogeochemical variables, 2) integrate measurements for a more robust prediction of bioluminescence water-leaving radiance (Moline *et al.* 2007; Oliver *et al.* 2007), 3) refine the use of bioluminescence as a measurement that can distinguish trophic groups, and 4) relate bioluminescent signals to taxonomically identified sampled organisms. The recent long-term data collected at Cal Poly highlights that bioluminescence, in conjunction with other IOPs can improve our understanding of ecosystem dynamics. It is anticipated that the data end products developed by our academic partners will aide and make the case for the transitioning the U-BAT package for routine deployment by the oceanographic community. We expect commercial availability of this instrument will greatly accelerate the general undestanding of marine bioluminescence.

## TRANSITIONS

All four prototype builds have been completed and a build for five additional units are underway as WET Labs move toward full commercial production. WET Labs will continue to make available U-BATs for

## RELATED PROJECTS

As the five additional units become available, our partnership with collaborators and the Navy will provide the opportunity to continue to test and define the operational capability of U-BAT. We view the final period of this award as the opportunity to seed the both research and Navy with U-BAT. Its acceptance by the first users is critical to overall success of the Phase II project and the commercial U-BAT product.